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Red Cell Blood Group Antigen Typing by DNA Microarray: Immucor PreciseType™ HEA Molecular BeadChip Test

Background Information

Testing of the red cell phenotype is often required for patients referred to the blood bank for pre-transfusion testing. The standard method of performing the red cell phenotype is by serology, using reagent antisera specific for the antigens of interest. However, serological phenotyping is labor-intensive, the antisera are expensive and may be in short supply, and the method is not suitable for all patients (e.g., recently transfused patients). The PreciseType™ HEA assay is a DNA-based test that overcomes some of the limitations of serological phenotyping. It predicts the probable red cell phenotype by detecting single nucleotide polymorphisms (SNPs) that define many blood group antigens.

Clinical Indications

The PreciseType™ HEA Assay is indicated for patient testing when a red cell phenotype is needed but serological phenotyping cannot be performed. Appropriate candidates for the assay include:

- Recently transfused patients or patients with a positive direct antiglobulin test,
- Patients at high risk for red cell alloimmunization, including patients with sickle cell anemia and other frequently transfused patients,
- Patients with auto-antibodies or other interfering reactivity that complicates phenotyping and antibody identification,
- Selected patients with Fyb-negative serological phenotype, for evidence of the Fyb silencing mutation (patients with this mutation generally do not require Fyb-negative red cell units for transfusion), and
- Patients who need to be typed for high-prevalence or low-prevalence antigens for which antisera are not readily available.

Blood centers can use the HEA assay to type blood donors for rare red cell phenotypes.

Methodology and Interpretation

For many red cell antigens (notably excluding the ABO and D antigens), alleleic differences generally result from SNPs, and detecting these SNPs provides the basis for determining antigen expression. The PreciseType™ HEA assay uses a DNA microarray to detect, in a single test, the polymorphisms associated with red cell antigens C/c, E/e, VS, V, K/k, Jsa/Jsb, Kpa/Kpb, Fya/Fyb, Jka/Jkb, M/N, S/s, U+/-, Lua/Lub, Doa/Dob, Hy, Joa, LWa/LWb, Dia/Dib, Coa/Cob and Sc1/Sc2. The assay also detects the following mutations that cause weak or silent antigen expression: 265C>T and -67T>C in the FY gene, and Intron 5 g>t and 230 C>T in the GYPB gene.

The PreciseType™ HEA assay is performed on DNA extracted from EDTA whole blood. The DNA region of interest is amplified by multiplex PCR, processed into single-stranded DNA and hybridized with allele-specific oligonucleotide probes to detect the relevant SNPs. The hybridization reaction is performed on a semiconductor chip mounted on a slide. Each chip contains beads expressing a library of allele-specific oligonucleotide probes matching either the wild-type or mutant allele. Hybridization of the test DNA with a matching probe results in probe elongation that is visualized by incorporation of a fluorescent label. An imaging system analyzes the intensities of the fluorescent signals for each SNP. Web-based software interprets the polymorphisms to determine genotype, and provides a report of predicted phenotype, with each antigen listed as '+' (present) or '0' (absent).

The PreciseType™ HEA assay has been approved by the U.S. Food and Drug Administration for determining non-ABO/non RhD red blood cell types for blood donors and patients. It has been validated for patient use by the Cleveland Clinic Section of Transfusion Medicine.

Limitations of the Assay

- The assay does not detect all clinically significant antigens.
- Assay results could be misleading, because the genotype may not always reflect the phenotype. Reasons for a genotype-phenotype discrepancy include rare polymorphisms that are not represented in the assay (e.g., those found in ethnic minority populations), and undetected silent phenotype due to mutations that is not analyzed by the assay. Discrepancies between the molecular type and the expressed phenotype must be investigated.
- Assay results should be interpreted cautiously for patients transfused with non-leukocyte-reduced blood, infants, hematopoietic stem cell transplant recipients and patients with severe leukopenia or leukocytosis.

References

1. Hashmi G et al. A flexible array format for large-scale, rapid blood group DNA typing. *Transfusion*. 2005;45:680.
2. Hillyer CD, Shaz BH, Winkler AM, Reid M. Integrating molecular technologies for red blood typing and compatibility testing into blood centers and transfusion services.*Transfus Med Rev*. 2008;22:117-132.
3. Klapper E et al. Toward extended phenotype matching: a new operational paradigm for the transfusion service. *Transfusion*. 2010;50:536.
4. Lomas-Francis C, DePalma H. DNA-based assays for patient testing: their application, interpretation, and correlation of results. *Immunohematology*. 2008:24:180-190.
5. Ribeiro KR et al. DNA array analysis for red blood cell antigens facilitates the transfusion support with antigen matched blood in patients with sickle cell disease. *Vox Sang*. 2009;97:147.
6. Sapatnekar S, Figueroa PI. How do we use molecular red cell antigen typing to supplement pre-transfusion testing? *Transfusion.* 2014;54:1452.
7. Westhoff CM. The potential of blood group genotyping for transfusion medicine. *Immunohematology*. 2008;24:190-195.

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Test Overview

Test Name	HEA Assay
Ordering Mnemonic	SQHEA
Specimen Requirements	EDTA whole blood (pink-top) tube, refrigerated. Blood bank requirements apply for sample collection, labeling and phlebotomist identification.
Clinical Information	This assay predicts the red cell antigen phenotype based on the patient's genotype. The test is interpreted by a pathologist in the context of the patient's clinical and immunohematological findings.
Disclaimers or Notations	The test results are not intended as the sole means for clinical diagnosis or patient management decisions. There are situations where the genotype of a person may not reflect the red cell phenotype. Mutations that inactivate gene expression or rare new variant alleles may not be identified in this assay.
Billing Code	87880
CPT Codes	81403, G0452

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